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Laboratory model for studying experimental fasciolopsiosis

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ABSTRACT. Eggs of Fasciolopsis buski for experimental examination of fasciolopsiosis were obtained from feces of artificially infected pig by several sieving and washing. Embryonic development, lasted 15 days at 30°C and hatching of miracidia was stimulated by low energy of light. Intermediate host, Segmentina hemisphaerula proved to be susceptible and the take varied between 25 and 46 per cent. Intramulluscan development lasted 60 days post infection at room temperature. Prepatent period was 50-65 days, peak of egg-production between 90-120 days (3 000-4 500 EPG) and later it decreased gradually.

KEY WORDS: Fasciolopsis buski (Trematoda), life cycle, Segmentina hemisphaerula (Mollusca)

Fasciolopsis buski (Lankester, 1857), the giant intestinal fluke, is a parasite of man and hog in several South - and South-East Asian countries. While particular attention has been paid to fasciolopsiosis of man in the endemic areas, fasciolopsiosis of pigs, however, had a relatively little room for examinations. Those examinations, dealing with the life-cycle of this fluke, have usually used metacercariae, derived from naturally infected snails. Thus, several methodological details were left out of considerations which are necessary to propagate metacercariae in appropriate time and quality for artificial infestations.

The present report is concerned with laboratory observations on the complete life-cycle of this fluke.

MATERIAL AND METHODS

The original stock of metacercariae of F. buski and that of the intermediate snail hosts (Segmentina hemisphaerula O.F. Müller, 1774) were collected by the author during his visit in Vietnam, Hanoi district. Three piglets, two months old, were infected with 1 000 metacercariae each, and they served as donors for further examinations.

Eggs were recovered from the fresh feces by dilution with water (1:4) and by subsequent sieving. The pore aperture of the last sieve was not more than 0.04 mm. The material retained in the sieve was washed with water into Petri dishes. Eggs were incubated in thermostate at 30°C.

Regular aquaria of 3 to 5 liters capacity, provided with airation, were used for breeding and holding the snails. Dechlorinated tap water was applied, it was changed twice a week. Newly hatdhed snails were fed on green algae, cultivated separately; older ones were fed on fresh or boiled and dried lettuce.

RESULTS AND DISCUSSION

Embryonic development

The eggs are light yellowish-brown in color. Measurements of eggs are 0.12-0.14 mm in length and 0.07-0.09 mm in width.

Freshly laid eggs usually contain zygote or blastule of a few cells. At first, the development is rather slow but later it becomes more rapid. During incubation at 30°C no significant change can be observed in the first 5-6 days, except for the growth of the embryo and its oval elongation. With the growth of the embryo, the vitelline cells gradually decrease in number and the place of the vitelline cells embracing the embryo is now occupied by big vacuoles. On the 10th and 12th days the embryo reaches a size of 120-150 µm and seems to be capable of hatching. In fact, however, hatching begins only later, as the miracidia has become capable of hatching not only morphologically but also physiologically. Hatching of miracidia usually begins on the 13th day and continues on the following one or two days. Hatching is stimulated by artificial illumination.

Miracidia

The hatched and free-swimming miracidium is fusiform with an X-shaped eye-spot, covered with cilia; an animalcule is 150-190 µm in length and 40-50 µm in width. The body of miracidia is covered with flat epidermal cells in five transverse rows, according to the formula 6:6:6:6:6. The life-span of miracidia kept in tap water at room temperature was 10-12 hours but their infectivity was limited to the first three hours only.

Intermediate snail host

Several sets of infections were performed with young (2-3 weeks old, 3-4 mm in diameter) snails, S. hemisphaerula, at room temperature (20-22°C). Eight-ten miracidia were added to snails kept in water individually in the hollows of a microtiter plastic plate. The take varied between 25 and 46 per cent. A total of 40 snails of the same age were cultivated in an aquarium to detect their egg-producing capacity. They produced an average of 0.4 egg masses per day. A count of 123 egg cluthes showed that the average number of eggs in an egg mass is 11-12. Accordingly, each snail produced 5-6 eggs per day on average. The incubation period of eggs varied from 5 to 7 days, the hatching rate varied from 93 to 100%. They matured in 25-30 days after hatching, when the shell diameter was 3-4 mm. Deposition of the eggs lasted up to three months or so. By this time 80% of the initial sum of snails died.

Intramolluscan larval stages

After penetration, the invading miracidia metamorphose into sporocyst. They grow rapidly until they reach an average size of 400 by 110 µm. The rediae, formed within the sporocyst, liberate by their rupture, in the lymphatic spaces. The rediae have a large digestive tract with a pharynx, birth pore and locomotor appendages. Rediae can be fully developed within 32-35 days after infection. First cercariae were liberated on the 35th day after infection. Newborn cercariae are poorly developed and migrate slowly to the hepatopancreas of the snail where the growth of their organs continues and they mature eventually. The first cercariae emerged on the 60th day after infection. Metacercariae are whitish in color, vigorously swimming organisms. When they were killed in hot water, their body was somewhat spearshaped or oval, measuring 180-195 by 150-158 µm while the tail was 490-520 by 52-59 µm. The emergence of cercariae was observed in the area which is contiguous with the anus of the snail. At this point a teat-like appearance can be seen and the cercariae extruded with considerable force during the processe. Emergence of cercariae showed an interesting periodicity. It took place in the evening and night hours (emergence began from 10 p.m. and continued through night up to 6-7 a.m. Almost fifty percent of the cercariae emerged between midnight and 2 a.m. After the emergence, cercariae swim away from the snail and they begin to encyst soon on the surface areas of the container. For the practical purpose, a sheet of cellofane being put on the water surface proved to be the best metacercarial trap. Metacercariae are spherical and have a diameter of 130-140 µm. The wall of the cyst is thin (7 µm) and thus the metacercariae are less resistant to dryness. Through the cyst-wall the

excretory bladder and ducts, filled with granules, are easily seen. The life-span of metacercariae was 70 days at room temperature and in moisture (NGUYEN THI LEE and SEY, 1985) but their infectivity considerably decreased after 45 days.

Development in definitive hosts

Our examinations embraced three pigs and ten rabbits as definitive hosts. They were fed on the metacercariae in gelatine capsule with the appropriate number of metacercariae (pigs - 500, rabbits - 200 each). All experimental animals became infected. Rabbits were sacrificed in every two months. It was observed that the fluke did not survive the infection in rabbits after ninety five days. In this age flukes were 15-17 mm in length having no eggs. Accordingly, rabbits did not seem to be model animals in a study of experimental fasciolopsiosis. WU (1937) and recently MALVIYA (1985), however, demonstrated, that F. buski can reach its maturity in rabbit, as some of these flukes contained eggs, but they did not test whether these eggs were really fertile or not. Our observations on the development of F. buski in pig were similar to those presented by BARLOW (1925), NAKAGAWA (1921), NAKAGAWA and SUZUKI (1923), YOUNG (1936), TRIPATHI et al. (1973) and NGUYEN THI LE and SEY (1985). The first eggs were discharged in 50-65 days after infection, but the greatest number of eggs (3 000-4 500 EPG) was layed in 90-120 days old infection and as time passed, the daily egg-production considerably decreased. When the last donor was sacrificed 370 days after infection, the take was 15 per cent and a few number of eggs were found in the uterus.

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